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Diversity of *Olea* genotypes and the origin of cultivated olives

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Abstract Tandem repeats belonging to three DNA sequence families (*Oe*Taq80, *Oe*Taq178, and *Oe*GEM86) were isolated from the nuclear DNA of *Olea europaea* cv. Carolea and dot-hybridized to the genomic DNA of 14 hypothetically different *Olea* species, 78 olive cultivars, and 14 wild olives. The copy number per unreplicated haploid genome of *Oe*Taq80- and *Oe*Taq178-related sequences was in the 10^7 –10^6 range and that of *Oe*GEM86-related sequences was in the 10^5 range in cultivars, wild olives and some *Olea* species. A large variation in the frequency of repeats belonging to each sequence family was observed within each group of plants. Positive correlations existed in each genome between the frequencies of repeats belonging to each family, and their overall frequency was positively correlated to the genome size. Duncan grouping showed that the frequency variation of tandem repeats within each group of plants was not continuous. Two main groups and several subgroups of genotypes could be separated within both the olive cultivars and the wild olives. Discrete areas in the Mediterranean Basin could be delimited by the geographic distribution of cultivated olives with different genotypes and the wild plants were associated with the cultivars in these areas according to genotypic similarity. The *Olea* species could be divided into four genotypic groups. Three of these, comprising accessions from Asia and North Africa, showed similarity with the genotypes of cultivars and wild olives. These results suggest a polyphyletic origin of cultivated olives from different wild *Olea* forms distributed throughout the Mediterranean Basin.

Keywords Genotype variation · *Olea* species · Olive cultivars · Tandem repeated DNA sequences · Wild olives

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Introduction

Olive (*Olea europaea* L.) is a long-living (at least several hundred years; Loussert and Brousse 1978), oil-producing plant, and the fruit is one of the oldest agricultural tree crops. Its cultivation most probably started in the third Millennium B.C. in the eastern region of the Mediterranean and later spread around the basin, where the species gained remarkable cultural and economic importance (Loukas and Krimbas 1983).

In spite of this long history, numerous important questions about the olive remain unanswered. Conflicting hypotheses exist about the phylogenetic relationships between *Olea europaea* and related *Olea* species (Chevalier 1948; Ciferri 1950; Zohary 1994). Many *Olea* forms have been ranked as species, but it is questionable as to how many of these deserve this rank (Green and Wickens 1989). The genetic relationships between cultivated olives and wild forms (*Olea* species and wild olive plants) are uncertain (Angiolillo et al. 1999). While up to 2,600 different olive cultivars have been recorded (Rugini and Lavee 1992), the biodiversity within *O. europaea* has mainly been described in terms of morphology and agronomic behaviour and, to date, the variability within *Olea* germplasm has been little explored. Some biochemical and molecular analyses have been carried out using isozymes (Ouazzani et al. 1993; Trujillo and Rallo 1995) or DNA polymorphisms (Fabbri et al. 1995; Amane et al. 1999; Angiolillo et al. 1999; Besnard and Bervillè 2000; Hess et al. 2000). However, we are far from having a suf-

Table 1 Olive cultivars, wild plants and *Olea* species studied

Origin	Material	Source[a]
	Cultivar	
North Italy	Casaliva	IRO
	Gargnà	IRO
	Taggiasca	IRO
Central Italy	Ascolana Tenera	IRO
	Dolce Agogia	IRO
	Frantoio	IRO
	Leccino	IRO
	Moraiolo	COGB
	Pendolino	IRO
	Seggianese	IRO
South Italy	Carolea	COGB
	Cima di Bitonto	IRO
	Coratina	COGB
	Oliva di Cerignola	IRO
	Uovo di Piccione	IRO
Italy-Sicily	Biancolilla	IRO
	Giarraffa	IRO
	Moresca	IRO
	Nocellara del Belice	IRO
	Passalunara	IRO
	Tonda Iblea	IRO
	Zaituna	IRO
Italy-Sardinia	Bosana	IRO
	Nera di Gonnos	IRO
France	Tanche	COGB
Central spain	Castellana	COGB
	Manzanilla Cacerena	COGB
	Verdial de Badajoz	COGB
South Spain	Gordal Sevillana	COGB
	Hojiblanca	COGB
	Lechin de Granada	COGB
	Lechin de Sevilla	COGB
	Manzanilla de Sevilla	COGB
	Picual	COGB
	Picudo	COGB
East Spain	Alfafara	COGB
	Arbequina	COGB
	Blanqueta	COGB
	Empeltre	COGB
	Salonenque	COGB
	Villalonga	COGB
Morocco	Picholine marocaine	COGB
Algeria	Kemlal de Kabylie	COGB
	Sigoise	COGB
Tunisia	Chemlali	COGB
	Chetoui	COGB
	Gerboui	COGB
	Marsaline	COGB
	Meski	COGB
	Ouslati	COGB
	Zalmati	COGB
Egypt	Doleci Hamed	COGB
Israel	Nabali	COGB
Lebanon	Souri	COGB
Syria	Chalchali	COGB
	Dam	COGB
Syria	Kaissy	COGB
	Sefraoui	COGB
	Sourani	COGB
	Zaity	COGB
Turkey	Ayvalik	COGB
	Domat	COGB
	Elmacik	COGB
	Izmir Sofralik	COGB
	Kiraz	COGB
	Memecik	COGB
	Uslu	COGB
Greece	Adramitini	COGB
	Amigdaloia	COGB
	Chalkidiki	COGB
	Kalamata	IRO
	Konservolea	COGB
	Koroneiki	COGB
	Mastoides	COGB
	Megaritiki	COGB
	Mirtolia	COGB
	Valanolia	COGB
Croatia	Crnica	COGB
	Wild olives	
South-east Italy	GA1	IRO
	MF1	IRO
	MF2	IRO
	MO1	IRO
Sicily (Italy)	AL1	IRO
	ME1	IRO
Sardinia (Italy)	BS2	IRO
	PC2	IRO
Corsica (France)	BO15	INRA
	OS4	INRA
Balearic Islands	*O. europaea* var *sylvestris*	KEW
Andalusia (Spain)	NH	COGB
Morocco	MA22	INRA
	MA23	INRA
	Olea species	
China	*O. cuspidata* Wall.	IRO
India	*O. ferruginea* Royale	IRO
Yemen	*O. chrysophylla* Lam.	INRA
Algeria	*O. laperrini* Batt. & Trab.	INRA
Canary Islands	*O. cerasiformis* Webb & Berth.	INRA
Ivory Coast	*O. capensis* Hochstetteri Bak.	INRA
Kenya	*O. africana* Mill.	KEW
	O. indica Klein	KEW
South Africa	*O. capensis* L.	INRA
	O. exasperata Jaq.	INRA
	O. woodiana Knobl.	INRA
Mauritius Island	*O. lancea* Lam.	KEW
Australia	*O. paniculata* R.Br.	KEW
Sumatram	*O brachiata* Merrill	INRA

[a] INRA, UR Genetique et Amelioration des Plantes, Montpellier, France; IRO, Institute of Olive Research, CNR, Perugia, Italy; COGB, Olive Germplasm Bank, Cordoba, Spain; KEW, RGB Kew Living Collection, London, UK

ficient understanding of the biodiversity among and within *Olea* species, olive cultivars and wild populations.

Repeated sequences constitute the major fraction of plant nuclear DNA, and a fraction of repetitive DNA is made up of sequences that are arranged tandemly in clusters. The repetition of these sequences varies greatly among species, with some variation within species. Moreover, the nucleotide sequence of these DNA repeats may change very rapidly due to their non-coding nature (Frediani et al. 1999, and references therein). Therefore, by comparing corresponding families of tandem repeated sequences, information may be obtained about the evolution and divergence of genotypes that could be useful for assessing phylogenetic relationships between species and intraspecific biodiversity

In the investigation reported here, the redundancy of tandem repeats belonging to three sequence families in the genome of *Olea* species, olive cultivars and wild olive plants was examined. The results show different genotypes within each of these groups of plants and provide us with some insight with respect to their interrelationships.

Materials and methods

Plant material and DNA extraction

Leaves were collected from 14 *Olea* accessions representing hypothetically different species, 78 olive cultivars, and 14 wild olives (Table 1). At least three individuals for each accession were sampled. Total DNA was extracted using the method previously described for olive (Angiolillo et al. 1999). Five grams of ground leaves was incubated with 2 × CTAB buffer for 1 h at 65 °C. Chloroform extraction was repeated twice, and RNA was removed from the aqueous solution by treatment with RNase (10 µg/µl) for 1 h. After isopropanol/ethanol precipitations, DNA was resuspended in TE buffer. About 20 µg DNA per gram of fresh tissue was obtained. To estimate the purity of DNA, we made readings on the spectrophotometer at wavelengths of 260, 280 and 230 nm; only values higher than 1.8 for OD_{260} /OD_{280} and 2.0 for OD_{260} /OD_{230} were accepted. In addition to spectrophotometric readings at 260 nm, DNA concentration was estimated by measuring the band intensity on ethidium bromide stained gels using the Gel Doc System (Bio-Rad, Hereovles, Calif.) for image acquisition and the Molecular Analyst Software (Bio-Rad) for quantification.

DNA fractionation and cloning, and probe selection

Genomic DNA was fractionated in two ways. (1) It was digested to completion (10 U/µg of DNA for 2 h at 37 °C) with *Alu*I (Roche) and fractionated by electrophoresis on a 1% agarose gel. The DNA present in a 21-kb band, which was visualized under UV light after ethidium bromide staining, was recovered from a preparative 2% agarose gel and digested with *Taq*I (Roche). The DNA fragments were cloned in pBS- *Acc*I/Bap. Two selected clones were sequenced and used as probes. (2) Genomic DNA was double-digested to completion as above with *Eco*RI and *Xba*I (Roche) and cloned in Lambda GEM-2 vectors (Promega, Madison, Wis). The DNA library was plated, and clones containing repetitive DNA were identified by plaque hybridization with genomic DNA which had been labelled with digoxigenin-11-dUTP (Roche) using a random-primed DNA labelling kit (Promega). The clone showing the highest hybridization signal was selected and used as a probe.

DNA sequencing

DNA sequencing was performed using the dideoxy method of Sanger et al. (1977) as modified by Chen and Seburg (1985) for double-stranded DNA. Computer analysis of the sequence data and sequence comparisons were carried out using the PC/Gene program (IntelliGenetics).

Dot-blot hybridization and calculation of sequence copy numbers

Replicate samples of 15, 30, 60 or 120 ng of genomic DNA and 10^7, 10^8 or 10^9 copies of the probe DNA sequences (dilution spots) were suspended in 10 µl of TE buffer (0.01 *M* Tris-HCl, pH 8.0, 0.001 *M* EDTA, pH 8.0) and applied to Zeta-probe (Bio-Rad) filters using a Minifold I apparatus(Schleicher & Schuell). Loading was controlled by adding lambda DNA to plant DNA. Hybridization was performed under high-stringency conditions (65 °C; 5 × SSC in the hybridization mixture), and each filter was probed in series with 50 ng per filter of repetitive DNA probes and lambda DNA that had been labelled with digoxigenin as described above. The filters were processed and scanned after hybridization as described previously (Ceccarelli et al. 1995). Digoxigenin haptens in DNA-DNA hybrids were detected using a Dig-DNA detection kit (Roche) by means of an enzyme-linked immunoassay with an antidigoxigenin-alkaline phosphatase conjugate.

The linear regression equation relating the natural logarithm of the copy number of probe sequences in the dilution spots and the natural logarithm of the corresponding densitometric readings was used to estimate the copy number of the sequences probed in the samples of genomic DNA.

The Duncan procedure of SAS version 6.03 (SAS Institute 1987) was used for statistical analysis of the data.

Results

Three tandem arrays of repeats were isolated from the nuclear DNA of *O. europaea* cv. Carolea. The first array contained seven repeats about 80 bp long (hereinafter termed *Oe*Taq80 repeats) sharing 74–91% nucleotide sequence similarity. It came available from a previous study in which the nucleotide sequence was shown (Bitonti et al. 1999). The second array was obtained by cleaving with *Taq*I the DNA of a high-molecular-weight band (about 21 kb) produced by electrophoresis of the genomic DNA digested with *Alu*I. It was made up of six repeats, all 178 bp long (*Oe*Taq178 repeats), with a minimum of 85% similarity (Fig. 1).The third array was isolated from an *Eco*RI+*Xba*I DNA library cloned in Lambda GEM-2 vectors. Its repeats were 85–86 bp long (*Oe*GEM86 repeats) and shared a minimum of 85% similarity. There was no obvious nucleotide sequence similarity among the three repeats, and they did not cross-hybridize. About 80% identity occurred between the *Oe*Taq80 repeats and *O. europaea* DNA elements of similar length previously described by Katsiotis et al. (1998). No other significant nucleotide sequence similarity was found after comparing *Oe*Taq80, *Oe*Taql78 or *Oe*GEM86 with DNA sequences in the PC/Gene program or in the EMBL/GenBank/DBJ nucleotide sequence databases.

The above repeats were dot-blot hybridized to the genomic DNA of *Olea* accessions representing hypothetically different species, a number of olive cultivars and

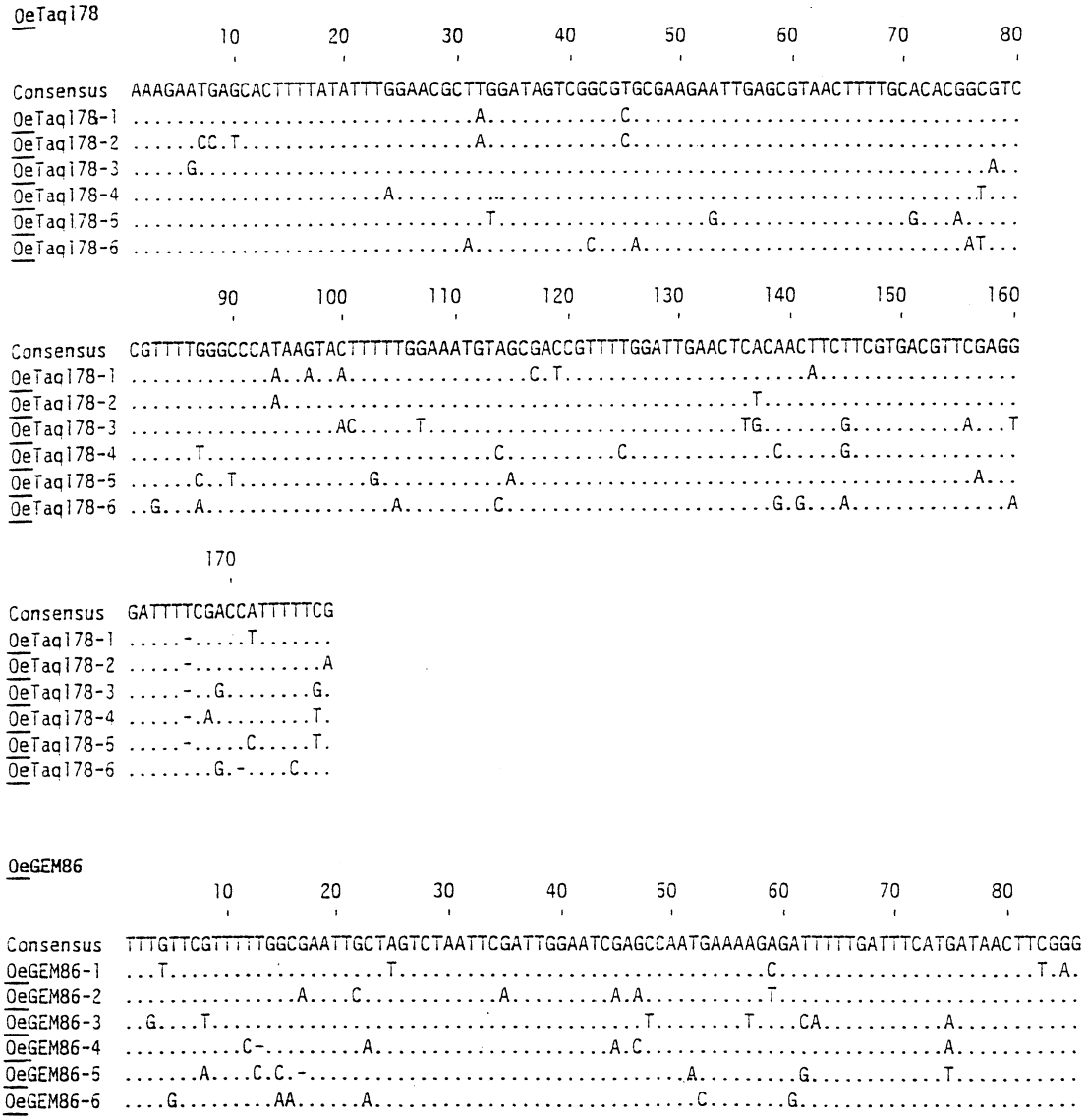

Fig. 1 Nucleotide sequences of six *Oe*Taq178 and six *Oe*GEM86 repeats in two tandem arrays from the genomic DNA of *Olea europaea* cv. Carolea. *Hyphens* represent gaps introduced to maximize homology. These sequences have been registered with EMBL Nucleotide Sequence Database under accession numbers AJ297958 and AJ297959, respectively

wild olive plants (Table 1). Linear values were obtained after scanning densitometrically the dot blots of 15, 30 and 60 ng of DNA of each genome (Fig. 2), and the copy number of sequences related to each DNA probe was calculated from these results. The frequency of *Oe*Taq80-related sequences was found to be in the 10^9 range per nanogram of genomic DNA and that of *Oe*Taql78-related sequences in the 10^8 –10^9 range in all of the olive cultivars and wild plants. *Oe*GEM86-related sequences were less represented (in the 10^7 range; Table 2, consisting of parts a, b and c).

Since the 1C DNA content of olive is about 2 pg (Rugini et al. 1996; Bitonti et al. 1999), the copy numbers of the *Oe*Taq80- and *Oe*Taq178-related sequences per unreplicated haploid genome were calculated to be in the 10^7 and 10^6 ranges, respectively, while that of *Oe*GEM86-related sequences was in the 10^5 range. Comparable frequencies of *Oe*Taq80-, *Oe*Taql78- and *Oe*GEM86-related sequences occur in a number of the *Olea* accessions studied. With other accessions, these frequencies decrease dramatically, and all sequence families are not detectable in certain accessions (Table 2, consisting of parts a, b and c).

Figure 3 shows that highly significant, positive correlations exist between the frequency of DNA sequences recognized by each probe in each genome. This suggests that changes in the three sequence families are under the same control and, consequently, the overall sequence frequency was used in subsequent data elaborations. Figure 4 shows that the copy number of the sequences is positively correlated with the genome size of the *Olea* species and olive cultivars in which the DNA content is known (Rugini et al. 1996; Bitonti et al. 1999). This suggests that the different levels of hybridization of the probes to different genomic DNAs are due to modula-

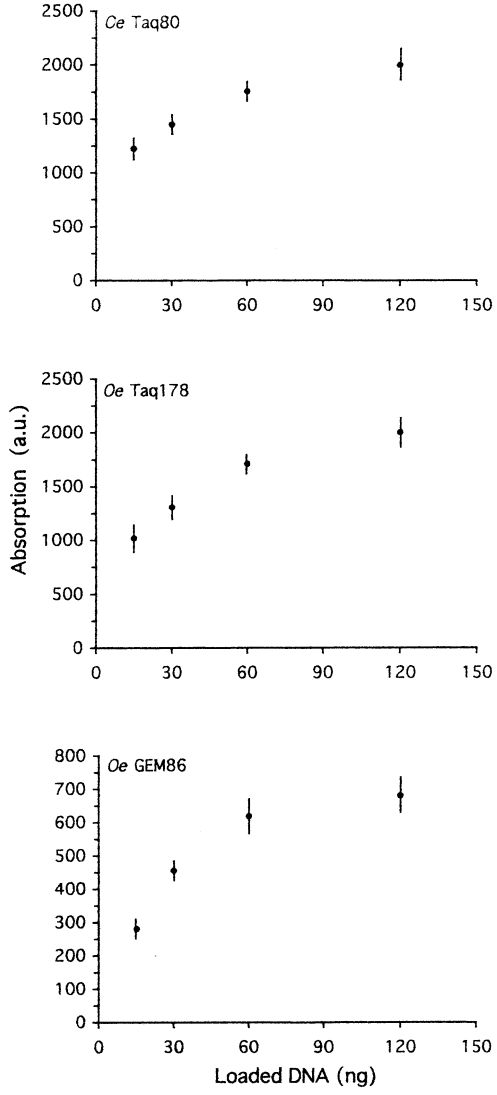

Fig. 2 Densitometric absorptions of dot-blot filters loaded with different amounts of genomic DNA from cv. Leccino and hybridized with different digoxigenin-labelled DNA probes. Each value is the mean of those obtained in six repetitions, three for each of two DNA extractions. Confidence limits. $P \leq 0.01$

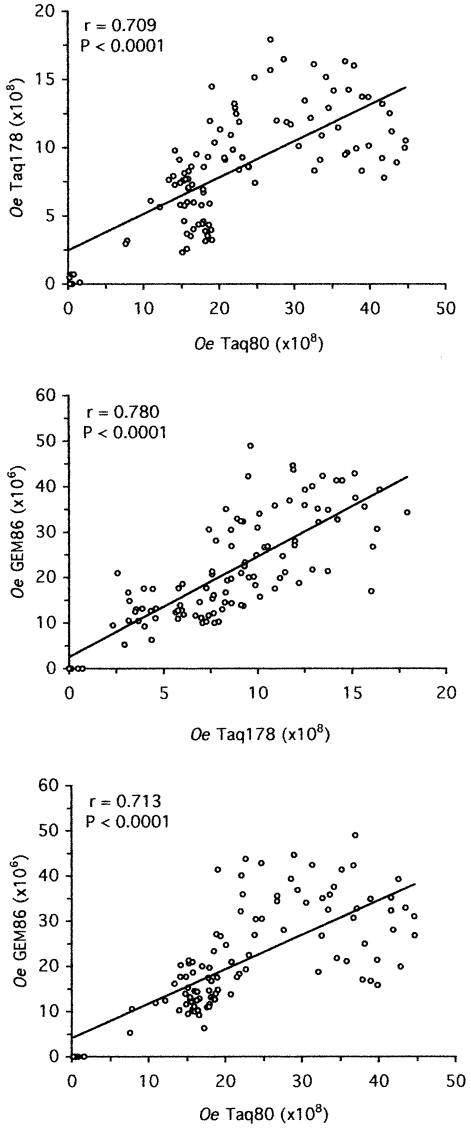

Fig. 3 Correlations between the frequency (copy number per nanogram of DNA) of tandem repeats belonging to different sequence families in each genome of olive cultivars, wild plants, and *Olea* forms

tions in the redundancy of repeated sequences rather than to their structural alterations.

Duncan grouping was used in Table 2 (consisting of parts a, b and c) to show that the large variation in the frequency of tandem repeats within both olive cultivars and wild plants is not continuous. Indeed, olive cultivars can be separated into two main groups, each of which is made up of subgroups. The overall frequency of tandem repeats ranges from about 40×10^8 to 55×10^8 copies per nanogram of DNA in the former group, and from 17×10^8 to 32×10^8 copies in the latter. The genotypes of the wild plants can be divided into two groups, a smaller group in which the frequencies of tandem repeats are in the range of those observed in the first group of cultivars (about 55×10^8 copies per nanogram of DNA), and a larger group where these frequencies are similar to those found

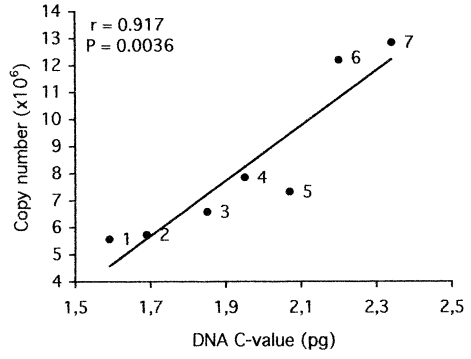

Fig. 4 Correlation between the overall copy number of *Oe*Taq80-, *Oe*Taql78-, and *Oe*GEM86-related sequences (Table 2, consisting of parts a, b and c) and the DNA content in the haploid (1C) genome of *Olea* forms and olive cultivars whose genome size is known (Bitonti et al. 1999). *1 O. africana*, *2 O. indica*, *3 O. ferruginea*, *4* cv. Dolce Agogia, *5 O. cuspidata*, *6* cv. Frantoio, *7* cv. Pendolino

Table 2a, b and c Copy number of tandem repeated sequences per nanogram of genomic DNA in olive cultivars, wild plants and *Olea* species as calculated from the results of dot-blot hybridizations with repeats belonging to three different sequence families. Each point is the mean (±SE) of the values obtained in six repetitions, three for each of two DNA extractions. Duncan grouping at $P \leq 0.01$ of the genomes according to the overall frequency of tandem repeats is shown

Material	*Oe* Taq80 ($\times 10^8$)	*Oe* Taq178 ($\times 10^8$)	*Oe* GEM86 ($\times 10^6$)	Overall frequency ($\times 10^8$)	Duncan grouping
Cultivars					
Frantoio	42.58±0.92	12.51±0.38	39.26±0.85	55.48	
Leccino	41.60±1.08	13.19±1.00	35.16±0.56	55.14	
Zaituna	42.83±0.82	11.18±1.06	19.88±0.52	54.20	
Moresca	37.88±0.99	16.04±1.19	16.98±0.88	54.08	
Lechin de Granada	39.78±1.19	13.72±1.08	21.36±0.81	53.71	
Passalunara	36.72±0.95	16.34±0.85	30.66±0.74	53.36	
Biancolilla	38.96±1.30	13.74±1.08	34.88±1.29	53.04	
Giarraffa	43.49±1.12	8.92±1.09	32.90±0.90	52.73	
Pendolino	37.18±0.96	14.26±1.08	32.72±1.31	51.76	
Ascolana Tenera	41.59±1.04	9.23±0.68	32.30±0.45	51.14	
Hojiblanca	39.85±1.29	10.13±0.99	15.78±0.73	50.13	
Domat	41.85±1.41	7.79±1.06	28.06±0.92	49.92	
Sigoise	35.18±1.23	14.19±1.18	41.34±1.16	49.78	
Elmacik	34.16±1.01	15.19±1.06	37.50±1.25	49.72	
Meski	32.57±1.13	16.11±1.19	26.76±1.07	48.94	
Villalonga	38.21±1.27	9.93±0.88	24.98±0.91	48.38	
Nocellara del Belice	34.54±1.24	12.91±0.78	21.76±0.65	47.66	
Alfafara	35.81±1.39	11.46±1.16	21.12±0.73	47.48	
Seggianese	38.93±0.88	8.30±0.50	16.74±0.60	47.39	
Manzanilla de Sevilla	36.98±1.31	9.63±0.88	49.00±1.39	47.10	
Salonenque	36.73±1.12	9.50±1.09	42.30±0.43	46.65	
Kiraz	28.59±1.33	16.49±1.16	39.30±0.99	45.47	
Zalmati	31.35±1.36	13.46±1.06	42.36±1.15	45.23	
Ouslati	26.79±1.28	17.94±1.17	34.32±1.07	45.07	
Kemlal de Kabylie	33.60±1.46	10.92±1.08	35.78±1.08	44.87	
Ayvalik	32.12±0.61	12.19±0.46	18.72±0.71	44.49	
Castellana	33.42±1.42	9.11±0.82	32.46±0.56	42.85	
Tanche	26.79±1.22	15.69±0.71	35.58±1.00	42.83	
Memecik	29.45±1.39	11.70±0.55	36.88±0.87	41.51	
Gerboui	32.64±1.48	8.32±0.88	35.08±0.74	41.31	
Chetoui	28.98±1.35	11.88±1.09	44.60±1.07	41.30	
Marsaline	30.53±1.35	10.11±1.18	34.02±1.01	40.98	
Dolce Agogia	24.70±1.00	15.15±0.56	42.84±1.19	40.27	
Chemlali	27.62±1.24	11.98±1.05	28.00±0.98	39.88	
Megaritiki	23.08±1.28	9.30±0.58	22.48±0.90	32.60	
Crnica	24.79±1.24	7.42±1.01	30.54±1.26	32.51	
Bosana	22.64±1.12	8.39±0.89	19.34±0.80	31.22	
Nera di Gonnos	20.79±1.12	9.13±1.06	20.98±0.80	30.12	
Dam	20.69±0.99	9.25±1.09	13.76±0.62	30.07	
Picholine marocaine	19.42±0.88	10.37±0.98	26.64±1.11	30.05	
Gordal Sevillana	17.90±0.85	6.93±0.47	14.62±0.80	24.97	
Oliva di Cerignola	17.92±1.03	6.70±0.69	11.60±0.84	24.73	

in the second group of cultivated olives (22–33×10^8 copies). Four groups can be distinguished within *Olea* species. One group includes *O. ferruginea*, *O. cuspidata*, *O. africana*, *O. chrysophylla* and *O. indica* (34–35×10^8 copies of tandem repeats per nanogram of DNA). *O. laperrini* (21×10^8 copies) and *O. cerasiformis* (11×10^8 copies) differ significantly between each other and from the above accessions. The frequencies of tandem repeats in all of these species are comparable to those observed in olive cultivars and wild olive plants. In contrast, the frequency of tandem repeats is much lower in the genomes of *O. capensis*, *O. exasperata*, *O. woodiana*, *O. lancea*, *O. paniculata* and *O. brachiata*, where *Oe*GEM86-related sequences or sequences related to both *Oe*GEM86 and *Oe*Taq178 could not be detected (Table 2, consisting of parts a, b and c).

Figure 5 shows the geographic distribution of the above-mentioned accessions of *Olea* species. The accessions from Asia and North Africa have frequencies of tandem repeats that are comparable with those found in olive cultivars and wild plants. One group of genotypes comprises Asiatic and north-eastern African *Olea*

Table 2b

	Oe Taq80 ($x10^8$)	*Oe* Taq178 ($x10^8$)	*Oe* GEM86 ($x10^6$)	Overall frequency ($x10^8$)	Duncan grouping
Picual	18.72±0.76	5.88±0.58	13.96±0.62	24.73	
Cima di Bitonto	14.15±0.88	9.79±0.96	20.24±0.98	24.14	
Doleci	14.75±0.79	9.13±0.86	13.90±0.51	24.01	
Picudo	16.44±0.84	7.27±0.74	10.24±0.40	23.81	
Taggiasca	15.93±0.80	7.71±0.69	10.00±0.46	23.74	
Kaissy	15.41±0.79	8.12±0.82	13.02±0.42	23.66	
Empeltre	17.66±1.03	5.77±0.50	10.98±0.48	23.53	
Kalamata	15.59±0.86	7.67±1.05	12.10±0.60	23.38	
Gargnà	15.98±0.59	7.07±0.68	10.00±0.51	23.15	
Uovo di Piccione	15.30±0.78	7.58±0.92	20.68±0.94	23.08	
Sefraoui	18.94±1.06	3.97±0.45	17.56±0.88	23.08	
Uslu	18.62±1.08	4.33±0.68	12.68±0.46	23.07	
Casaliva	15.92±0.51	7.02±0.53	11.12±0.36	23.05	
Arbequina	15.25±0.89	7.59±0.31	21.26±0.82	23.05	
Manzanilla Cacerena	15.15±0.68	7.60±0.87	15.32±0.78	22.90	
Lechin de Sevilla	16.63±0.62	5.97±0.37	12.78±0.28	22.72	
Blanqueta	17.91±1.11	4.58±0.21	11.02±0.37	22.60	
Adramitini	19.05±1.16	3.22±0.12	14.82±0.72	22.41	
Chalchali	17.79±1.01	4.43±0.14	17.46±0.95	22.39	
Nabali	14.85±0.52	7.41±0.90	11.66±0.61	22.37	
Mirtolia	18.16±1.05	3.88±0.22	13.16±0.67	22.17	
Zaity	18.50±1.01	3.55±0.17	12.98±0.49	22.17	
Mastoides	15.79±0.98	6.01±0.53	18.62±0.54	21.98	
Izmir Sofralik	17.26±1.00	4.37±0.19	6.32±0.31	21.69	
Verdial de Badajoz	14.14±0.78	7.28±0.95	17.66±0.67	21.59	
Chalkidiki	18.19±0.87	3.15±0.14	16.76±0.72	21.50	
Valanolia	15.41±0.69	5.76±0.46	12.70±0.46	21.29	
Koroneiki	14.87±0.52	5.80±0.52	17.68±0.83	20.84	
Coratina	16.60±0.81	4.01±0.29	9.26±0.36	20.70	
Sourani	15.37±0.60	4.61±0.35	13.12±0.56	20.11	
Souri	16.26±0.77	3.51±0.29	12.48±0.72	19.89	
Moraiolo	15.72±0.50	3.69±0.12	10.44±0.43	19.51	
Amigdaloia	15.72±0.88	2.57±0.16	20.94±0.95	18.49	
Carolea	12.14±0.74	5.64±0.46	12.42±0.83	17.90	
Konservolea	15.13±0.82	2.33±0.17	9.50±0.50	17.55	
Tonda Iblea	10.91±0.47	6.08±0.38	11.84±0.44	17.10	
Wild olives					
AL1	44.69±1.35	10.54±0.57	26.82±1.17	55.49	
ME1	44.61±1.43	10.00±0.62	31.00±1.34	54.92	
BO15	23.95±1.20	8.59±0.42	30.46±1.24	32.84	
BS2	23.83±1.16	8.61±0.53	26.90±1.23	32.70	
GA1	21.53±1.06	10.93±0.77	17.60±0.93	32.63	
MO1	21.80±1.01	9.87±0.61	18.32±0.95	31.85	
OS4	20.11±1.07	11.33±0.79	24.72±1.18	31.68	
PC2	18.82±0.75	11.96±0.83	27.08±1.21	31.05	
NH	18.53±0.91	9.32±0.57	23.34±1.18	28.08	
MA22	17.96±0.82	8.59±0.68	19.66±0.91	26.74	
O. europaea var. *sylvestris*	17.01±0.90	9.52±0.69	20.04±1.11	26.73	
MA23	16.35±0.85	8.61±0.74	14.36±0.67	25.10	
MF1	15.98±0.76	8.26±0.56	14.52±0.74	24.38	
MF2	13.96±0.73	7.93±0.48	10.24±0.61	21.99	

Table 2c Legend see page 1234

Olea species	*Oe* Taq80 (x10^8)	*Oe* Taq178 (x10^8)	*Oe* GEM86 (x10^6)	Overall frequency (x10^8)	Duncan grouping
O. ferruginea Royale	22.02±1.08	13.24±0.60	32.16±1.21	35.58	
O. cuspidata Wall.	22.10±1.02	12.91±0.55	40.06±1.30	35.41	
O. africana Mill.	22.26±1.12	12.49±0.43	35.92±1.11	35.10	
O. chrysophylla Lam.	22.67±1.11	11.90±0.37	43.72±1.38	35.00	
O. indica Klein	19.06±0.94	14.49±0.43	41.34±1.37	33.96	
O. laperrini Batt. & Trab.	13.35±0.62	7.66±0.35	16.12±0.79	21.17	
O. cerasiformisWebb & Berth.	7.71±0.35	3.05±0.20	7.91±0.43	10.83	
O. lancea Lam.	1.60±0.18	0.10±0.01	0.04±0.005	1.74	
O. exasperata Jaq.	0.78±0.08	0.71±0.11	0.00±0.00	1.49	
O. capensis L.	0.49±0.03	0.71±0.11	0.00±0.00	1.20	
O. capensis Hoch. Bak.	0.20±0.02	0.48±0.07	0.00±0.00	0.68	
O. woodiana Knobl.	0.56±0.06	0.00±0.00	0.00±0.00	0.56	
O. paniculata R.Br.	0.48±0.03	0.00±0.00	0.00±0.00	0.48	
O. brachiata Merrill	0.26±0.00	0.00±0.00	0.00±0.00	0.26	

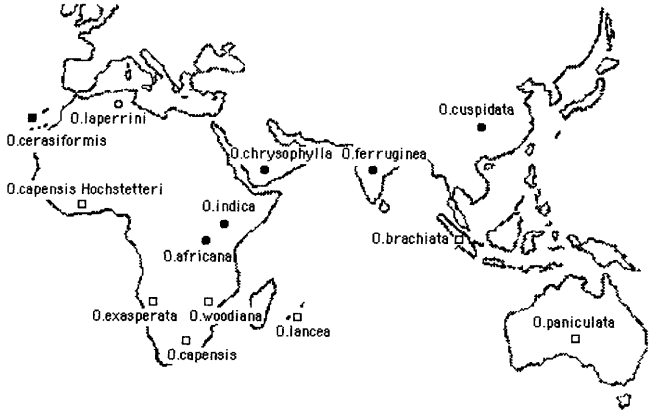

Fig. 5 Geographic distribution of the accessions of *Olea* forms studied. Accessions which differ significantly in the frequency of tandem repeated DNA sequences in their genomes (Table 2, consisting of parts a, b and c) are indicated by different symbols. ● 33.96–35.58×10^8 copies per nanogram of genomic DNA, ○ 21.17×10^8 copies, ■ 10.83×10^8 copies, □ 0.26–1.74×10^8 copies

Fig. 6 Distribution around the Mediterranean Basin of the olive cultivars (●, ○) and wild plants (■, □) studied. *Solid or open symbols* differentiate two main groups of accessions, which differ significantly in the frequency of tandem repeated DNA sequences in their genomes (Table 2, consisting of parts a, b and c); *solid symbols* 39.88–55.48×10^8 copies per nanogram of genomic DNA, *open symbols* 17.10–32.60×10^8 copies)

species. *O. laperrini* and *O. cerasiformis* are from northwestern Africa and the Canary Islands, respectively. The accessions whose genotypes differ largely from those of the olive cultivars and wild plants are from South Africa, the Mauritius Islands, Australia and Sumatra.

The geographic distribution of olive cultivars and wild plants having different genotypes (Table 2, consisting of parts a, b and c) is shown in Fig. 6. The distribution is not random; there are discrete areas in the Mediterranean Basin where cultivar genotypes are similar. Even if this does not appear from Fig. 6, cultivars belonging to each subgroup in Table 2 (consisting of parts a, b and c) are from definite regions. For example, cvs. Chemlali, Chetoui, Gerboui, Marsaline, Ouslati and Zalmati, all from Tunisia, belong to the subgroup where the overall frequency of tandem repeats is 40–46×10^8 copies per nanogram of DNA. Also the geographic distribution of wild olive plants having different genotypes is not random. As can be seen in Fig. 6, wild plants are grouped with cultivars according to genotype similarity in the discrete areas of the Mediterranean Basin.

Discussion

Our findings show that *Olea* genotypes can be differentiated based on the redundancy of tandem repeats in the nuclear DNA (Table 2, consisting of parts a, b and c). Changes in the redundancy of repeated DNA sequences are known to be powerful factors of species evolution. They differentiate related species (e.g. Narayan and Rees 1976) as well as populations, cultivars or lines within species (Bennett and Leitch 1995; Frediani et al. 1999). The correlation that we found between genome size and

overall redundancy of DNA repeats in *Olea* species and olive cultivars (Fig. 4), as well as between the redundancy, in each genome, of repeats belonging to different families (Fig. 3), is evidence of the reliability of our results. Another proof of reliability is that our grouping of hypothetically different *Olea* species agrees perfectly with the systematics of the *Olea* complex as proposed by Green and Wickens (1989) on the basis of morphological, karyological, anatomical, palynological and biochemical (flavonoids) evidence. These authors consider wild olives and olive cultivars, which share comparable genotypes according to our data, to be two varieties of the *europaea* subspecies, and rank the Asiatic and northeastern African forms, which have similar genotypes according to our results (*O. ferruginea*, *O. cuspidata*, *O. africana*, *O. chrysophylla* and *O. indica*; Table 2, consisting of parts a, b and c), under the subsp. *cuspidata*. The close affinity of these forms has also been recognized by Mazzolani and Altamura Betti (1978) and Angiolillo et al. (1999). Green and Wickens (1989) consider *O. laperrini* and *O. cerasiformis* to be two different subspecies, and our results indicate that they have different genotypes (Table 2, consisting of parts a, b and c). Hess et al. (2000) reached similar conclusions after comparing the genome of *Olea* forms using ribosomal internal transcribed spacer sequences, random amplified polymorphic DNAs, and intersimple sequence repeats as molecular markers. Moreover, the data from Green and Wickens (1989) and Angiolillo et al. (1999) agree with ours in showing the genetic distance between *O. brachiata*, *O. capensis*, *O. lancea* and *O. paniculata* and the North African and Asiatic *Olea* forms.

In agreement with other results to be found in the literature (Angiolillo et al. 1999, and references therein) our results indicate that genotypic variation occurs within both olive wild plants and cultivars (Table 2, consisting of parts a, b and c). It has already been shown that changes in the copy number of repeated sequences in the nuclear DNA can occur rapidly in a given species as a factor of environmental adaptation (Ceccarelli et al. 1997; Caceres et al. 1998). However, our findings show that the redundancy of tandem repeated DNA sequences does not differ between *O. ferruginea*, *O. cuspidata*, *O. africana*, *O. chrysophylla* and *O. indica* (Table 2, consisting of parts a, b and c), although the plants originate from China, India, Yemen, and Kenya (Table 1). Therefore, we consider the changes observed in DNA sequence organization to represent stable genotypic diversifications developed during species evolution, rather than rapid, environmentally controlled genomic modulations. Consequently, both wild olives and cultivars may be considered to be complexes of different genotypic entities.

We found that olive cultivars show genotypic affinity based on their area of cultivation (Fig. 6). Which is in agreement with the recognized existence of different ecotypes of cultivated olives (Ciferri 1950). Moreover, different groups of cultivars share genotypic similarity with different Afro-Asiatic *Olea* forms (Table 2, consisting of parts a, b and c). Angiolillo et al. (1999) also have shown that Afro-Asiatic *Olea* forms share genomic affinities with cultivated olives on the basis of results from amplified fragment length polymorphism analyses of nuclear DNA, even if these results failed to show any particular affinity of olive cultivars based on their area of cultivation. Our findings suggest a polyphyletic origin of cultivated olives. Different cultivars may have derived from, or have introgressed DNA elements of, different *Olea* entities distributed throughout the Mediterranean Basin, which would represent the stock from which the fruit trees cultivated in a given area are derived (Zohary and Hopf 1994). Data on DNA content and the spatial organization or the amount of heterochromatin in cell nuclei also support this view (Bitonti et al. 1999), and multiple domestications of olive trees are suggested by other, recent results obtained from molecular analyses of nuclear and mitochondrial DNA (Besnard and Bervillè 2000; Hess et al. 2000).

Therefore, as already suggested by Angiolillo et al. (1999), there was no unidirectional flux of domesticated olives from the eastern to western Mediterranean regions (Loukas and Krimbas 1983). Findings from archeological and historical studies have shown that olive cultivation most probably began in the Middle East and then expanded westward (Zohary and Spiegel-Roy 1975; Simmonds 1976; Loussert and Brousse 1978). However, it may well be that the spread of the olive culture (the idea and custom to cultivate olive trees) was not necessarily accompanied by the contemporary propagation of plants to be cultivated. Local wild forms were selected and cultivated, or crossed to improve agronomically interesting characteristics of olive trees. Our finding that olive cultivars and wild plants which have the same geographical origin also have correlated genotypes (Fig. 6) is in line with this assumption. A close correlation between wild olives and cultivars has also been suggested after a comparison of their nuclear, mitochondrial and chloroplast DNA polymorphisms (Amane et al. 1999; Besnard and Bervillè 2000; Hess et al. 2000) and leaf allozyme variation (Ouazzani et al. 1993). However, amplified fragment length polymorphism of nuclear DNA suggested that wild plants from Sicily evolved separately from Sicilian cultivated varieties (Angiolillo et al. 1999). Further study is in progress to better assess the phylogenetic relationships between wild olives and cultivars.

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